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MINIREVIEW

Role of the Laboratory in the Diagnosis of Enterohemorrhagic *Escherichia coli* Infections

Sue C. Kehl*

Department of Pathology, Medical College of Wisconsin, and Children's Hospital of Wisconsin, Milwaukee, Wisconsin

Shiga-toxin-producing *Escherichia coli* (STEC) organisms were first associated with enteric disease in the early 1980s when Riley et al. (26) reported on the isolation of *E. coli* O157:H7 from patients who experienced hemorrhagic colitis associated with the ingestion of undercooked hamburgers at a fast-food chain. At the same time, Karmali et al. (15) identified a fecal cytotoxin and fecal cytotoxin-producing *E. coli* in patients with hemolytic uremic syndrome (HUS). Konawalchuk et al. had previously identified this cytotoxin, produced by some strains of *E. coli*, in 1977 (17), when they reported an irreversible cytopathic effect on Vero cells quite distinct from the toxic effect of *E. coli* heat-labile toxin. These first reports identified an emerging class of enteric pathogens associated with gastrointestinal disease and renal complications. Many excellent review articles have been written on these organisms (12, 14, 21, 23, 25), and I will not duplicate their efforts here. This article will present a brief overview of the pathogenesis of enterohemorrhagic *E. coli* (EHEC), highlighting the numerous virulence factors these organisms possess and the increasing role of non-O157 EHEC in gastrointestinal disease and renal complications. I will discuss laboratory methods of detection and attempt to convince the reader of the necessity for the employment of virulence factor-based methods of detection.

Clinical manifestations of EHEC infection range from asymptomatic carriage to diarrhea to hemorrhagic colitis. HUS is a common complication in children; thrombotic thrombocytopenic purpura (TTP) is an infrequent complication in adults. In addition to patient age, a high white cell count (19) and treatment with antibiotics (30) are also predictive for the development of HUS.

Most EHEC are acid resistant, which allows them to survive the acid conditions of the stomach. It is believed that they adhere to the colon and distal small intestine; however, typical lesions have not been demonstrated. The best-characterized adherence phenotype is the intimate or attaching and effacing adherence (A/E). A/E lesions consist of loss of enterocyte microvilli and intimate attachment of the bacterium to the cell surface with associated cytoskeletal changes resulting in the formation of a pedestal. The mechanism whereby A/E lesions are produced is less characterized than that for enteropathogenic *E. coli* but is analogous. STEC organisms that demonstrate the A/E phenotype contain a LEE (locus for enterocyte effacement) pathogenicity island, which contains *eaeA*, whose gene product, intimin, mediates intimate attachment to the enterocyte. LEE also encodes a Tir (translocated intimin receptor) homolog which is secreted from the organism and delivered to the host cell along with a number of other proteins via a type III secretion system. Although there is a strong association between the presence of *eaeA* and severe disease, some STEC organisms do not possess the *eaeA* gene, including some from patients with HUS and hemorrhagic colitis. Thus, intimin is not essential for virulence (25).

STEC isolates that possess the *eaeA* gene are capable of producing diarrhea. However, the pathological lesions associated with hemorrhagic colitis and HUS are due to the action of Shiga toxins (STX) with endothelial cells. Shiga toxin 1 (Stx1) is identical to that produced by *Shigella dysenteriae*. Shiga toxin 2 (Stx2) is 56% homologous to Stx1. Bacteriophage contain the genes for toxin production; organisms which become lysogenic for these phage acquire the capacity to produce the toxins. Shiga toxins are composed of five B subunits and a single A subunit. The B subunit binds to globotriaosylceramide (Gb_3) receptor. The A subunit cleaves an adenine residue from the 28S rRNA component of the 60S eukaryotic ribosomal complex, blocking protein synthesis (23). STX is translocated from the apical surface of the enterocyte to the basolateral side in an energy-dependent fashion without damage to the enterocyte (21). In the bloodstream, STX targets tissues with the appropriate glycolipid receptor, the intestine, the central nervous system, and the kidney. Particularly high levels of human Gb_3 are found in the human kidney, particularly in the cortical region. Damage to glomerular endothelial cells results in decreased capillary blood flow and renal insufficiency (25).

Other accessory virulence factors, such as enterohemolysin and serine protease EspP encoded on the pO157 virulence plasmid, as well as host inflammatory mediators, interleukin 1β and tumor necrosis factor alpha, probably play a role in the wide variations in disease presentation (25). While STX mediates HUS in the vast majority of infections due to STEC, there have been rare reports of STX-negative *E. coli* O157:H7/H$^-$ being isolated from patients with HUS (27).

With the emergence of *E. coli* O157, diagnostic tests became available to facilitate detection of this organism. Most of these tests relied on the unique biochemical properties of *E. coli* O157, i.e., the inability to ferment sorbitol or to produce β-glucuronidase.

Sorbitol MacConkey agar (sMac), which contains 1% sorbi-

* Mailing address: Department of Pathology, Medical College of Wisconsin, 9200 W. Wisconsin Ave., P.O. Box 26509, Milwaukee, WI 53226-0509. Phone:(414) 266-2529. Fax: (414) 266-2779. E-mail: kskehl@mcw.edu.

TABLE 1. Selected outbreaks due to *E. coli* O157:H7

Location(s) (reference)[a]	Date (mo/yr)	No. of cases	No. (%) with HUS	Mortality (%)	Type of outbreak	Source
Oregon	2/82	26	0	0	Community	Hamburger
Michigan	5/82	21	0	0	Community	Hamburger
Nebraska	9/84	34	1 (2.9)	4 (11.8)	Nursing home	Hamburger
Ontario	9/85	73	12 (16.4)	19 (26.0)	Nursing home	Sandwiches
Ontario	4/86	30	3 (10.0)	0	School	Raw milk
Washington	10/86	37	4 (10.8)	2 (5.4)	Community	Ground beef
Utah	6/87	51			Custodial home	Ground beef
Minnesota	10/88	54	0	0	School	Ground beef
Missouri	12/89	243	2 (0.8)	4 (1.6)	Community	Water
North Dakota	7/90	65	2 (3.1)	0	Community	Roast beef
Oregon	7/91	21	3 (14.3)	0	Community	Water
Washington (5)	12/92	477	30 (6.3)	3 (0.6)	Community	Ground beef
Idaho (5)	12/92	14	1 (7.1)	0	Community	Ground beef
California (5)	12/92	34	7 (20.6)	1 (2.9)	Community	Ground beef
Nevada (5)	12/92	58	3 (5.2)	0	Community	Ground beef
Washington (6)	11/94	20	1 (5.0)	0	Community	Dry-cured salami
California (6)	11/94	3	0	0	Community	Dry-cured salami
Washington, British Columbia, California, and Colorado (9)	10/96	28	12 (42.9)	0	Community	Unpasteurized commercial apple juice

[a] Unless otherwise indicated, data are taken from references 14 and 17.

tol instead of 1% lactose, utilizes the inability of most strains of *E. coli* O157 to ferment sorbitol to improve detection. Early in the course of the disease, within 1 to 2 days of the onset of diarrhea, sMac culture detects organisms from most patients with infections caused by sorbitol-negative *E. coli* O157. As the disease progresses, the detection rate falls to 33% (21). Several studies have demonstrated an overall low sensitivity (50 to 60%) of sMac plates for the detection of non-sorbitol-fermenting *E. coli* O157 (16, 22). Recovery can be improved by selective broth enrichment or by the addition of cefixime and tellurite to sMac agar (21). In addition to non-O157 isolates not being detected, tellurite-sensitive *E. coli* O157and sorbitol-fermenting *E. coli* O157 have been reported and are also not detected by these methods (27).

The inability of *E. coli* O157 to produce β-glucuronidase is used by Rainbow agar, a chromogenic agar for the selection of *E. coli* and detection and identification of *E. coli* O157. In one study, while all non-O157 *E. coli* were recovered, additional testing was necessary for identification as Shiga toxin producers; only 25% of the *E. coli* O157 were the proper color for identification without additional testing (22).

Serotype-specific enzyme immunoassays are available for the detection of *E. coli* O157 directly from stool samples. These assays have sensitivities of 73 to 100% compared to sMac, an already insensitive test (10, 18, 24, 29). An advantage is that the tests can be performed directly from stool specimens; the disadvantages are that they detect *E. coli* O157 only, have poor sensitivity, and are costly.

PCR assays for Shiga toxins, multiplex PCR assays for other virulence factors including intimin and enterohemolysin, and DNA probes are sensitive tests often performed in individual laboratories and not widely available. Cytotoxin testing requires tissue culture capabilities and is difficult, time consuming, costly, and not routinely performed.

There are commercially available assays for the detection of Shiga toxins. These assays can be performed with stool specimens directly or after overnight broth enrichment. They are more sensitive than sMac, with reported sensitivity levels (82 to 100%) comparable to that of cytotoxin testing (16, 18, 22). The advantages of these tests are that they are not serotype specific, will detect all serotypes of STEC, and are faster and easier than cytotoxicity testing; a disadvantage is the cost.

There is compelling public health evidence to support the incorporation of routine testing for EHEC in the laboratory. Reports of both sporadic disease and outbreaks due to *E. coli* O157 have been increasing since it was first identified as a pathogen in the early 1980s. Table 1 lists selected outbreaks due to *E. coli* O157:H7 from which much of the spectrum of disease and the epidemiology were discerned. HUS and TTP are seen as common complications in some outbreaks. The percentage of individuals who develop HUS or TTP varies with the population involved. As can be seen in Table 1, the highest percentage of HUS is seen in outbreaks in which predominantly children or the elderly are infected. In addition to outbreaks, the number of sporadic cases of disease was also increasing. Prevalence studies highlighted the increasing incidence of *E. coli* O157:H7 infections that occurred over a short period of time. In the early 1980s, an incidence of 0.07% was reported from Chicago area hospitals (14), while 10 years later, studies reported incidence rates of 1.3 to 2.9% (1, 3). Sporadic infections appear to be more common in Canada than in the United States, while within the United States infections appear to be more common in northern than in southern states. *E. coli* O157 are also important pathogens in Europe and Japan (21).

Reports of outbreaks due to non-O157 EHEC are also on the increase. Prior to 1991, no outbreaks due to non-O157 EHEC had been reported in the United States, Canada, or the United Kingdom. Sporadic infection was also uncommon, although the lack of a simple test for the detection of these organisms may have led to its lack of recognition (12). As techniques have developed for the detection of non-O157

TABLE 2. Outbreaks due to non-O157:H7 EHEC

Location (reference)	Date (yr)	Organism	No. of cases	No. (%) with HUS	Mortality (%)	Type of outbreak	Source
Italy (4)	1992	*E. coli* O111:NM	NR[a]	9	1	Community	Ground beef
Montana (8)	1994	*E. coli* O104:H21	18	0	0	Community	Milk
South Australia (7)	1995	*E. coli* O111:H-	NR	21	0	Community	Mettwurst
Japan (13)	1996	*E. coli* O118:H2	128	0	0	School	Salad
Connecticut (20)	1999	*E. coli* O121:H19	11	3 (27.3)	0	Community	Lake water

[a] NR, not reported.

EHEC, infections associated with these organisms are being reported with increasing frequency. In the early 1990s, reports of outbreaks due to non-O157 EHEC started to appear in the literature (Table 2). Non-O157 EHEC may play a more important role in disease than *E. coli* O157 in Argentina, Australia, Chile, and South Africa (21).

Food is the primary source of the organism in these outbreaks, with cattle being the primary food source. High rates of bovine carriage of STEC have been reported from many countries, with non-O157 reported at a much higher prevalence rate than O157 STEC. These high rates in animals correspond to high rates in retail meats. In a survey of fresh meats and poultry in the United States and Canada, *E. coli* O157 was recovered from 4% of beef samples, 1.5% of pork, 1.5% of poultry, and 2.0% of lamb. However, in another U.S. study, no *E. coli* O157:H7 organisms were recovered from retail meats, but a high prevalence of non-O157:H7 STEC was found in beef (23%), pork (4%), lamb (48%), veal (63%), and chicken (12%) (14).

The increasing incidence of *E. coli* O157 infection seen in the 1980s and the serious complications associated with it prompted the Centers for Disease Control and Prevention (CDC) (5) in 1993 to recommend culture of all stools or, minimally, bloody stools using sMac agar. Recent studies employing virulence factor-based testing report that the prevalence of STEC is 1.5 to 4.0%, with non-O157 accounting for 25 to 50% of the isolates. These compare to rates of 2.6 to 3.4% for *Salmonella*, 0.2 to 3.1% for *Shigella*, and 0.9 to 2.5% for *Campylobacter* (Table 3) (1, 3, 16). This high prevalence of non-O157 infection and its association with serious complications combined with the high prevalence of non-O157 in animals and retail meats strongly support the utilization of virulence factor-based testing for detection of all serotypes of EHEC.

Incorporation of this testing into the routine microbiology laboratory comes at a significant additional cost. When the laboratory is also faced with significant pressure to contain or reduce laboratory costs, this becomes a difficult choice. With increasing emphasis on cost containment, laboratories may want to limit testing to those at highest risk. The CDC (5) recommends that testing be performed at least on bloody stool specimens. In the routine laboratory, only the presence of gross blood in the stool specimen not submitted in transport medium would qualify for testing. History of blood in the stool is clinical information often not conveyed to the laboratory, and the presence of blood in the stool may not be obvious in the stool specimen submitted in transport medium. One study reports that if the presence of gross blood were used as a criterion for testing, 3% of the stool specimens would be cultured, with detection of 63% of the isolates (28). However, another investigator found that the presence of blood had a positive predictive value of only 7% for the presence of STEC in that study (16). If the presence of gross blood had been used as a criterion for testing in that study, 6% of the specimens would have been cultured but only 25% of the STEC would have been identified.

Limiting the performance of EHEC testing to those at highest risk of complications has also been suggested. As evidenced in outbreaks, children and the elderly are at highest risk for these complications. Age-specific prevalence rates of HUS are difficult to find; however, a study in Minnesota in 1988 reported a rate of 5.8 per 100,000 children less than 5 years old compared to 0.41 to 1.15 per 100,000 children aged 5 to 15 years old (19). Testing only children less than 5 years old would have missed 50% of the STEC detected in one study (16). Detection of outbreaks would then occur only after the most serious cases had been identified.

Limiting EHEC testing based on local prevalence may also be useful. In some locations, the prevalence was found to be too low to justify testing. However, in most cases, once laboratories started testing for these organisms, reporting increased (21).

Certainly, once the decision is made to test for the presence of EHEC, virulence factor-based testing should be employed. The low sensitivity of the sMac agar culture and other serotype-specific methods argues strongly against their use, despite the low cost. Laboratories need to develop criteria to allow for the cost-effective implementation of these tests into the routine

TABLE 3. Prevalence of non-O157 EHEC

Location (reference)	Date (mo/yr)	Method of EHEC detection	Non-O157 EHEC (%)	*E. coli* O157:H7 (%)	*Salmonella* (%)	*Shigella* (%)	*Campylobacter* (%)
Seattle (3)	1/91–1/92	Probes for virulence factors	1.1	2.9	3.4	0.2	2.5
Rhode Island (1)	5/91–4/92	Fecal cytotoxin assay	1.3	1.3	2.6	2.2	NR[a]
Wisconsin (16)	12/93–11/94	Shiga-toxin EIA[b]	0.3	1.2	2.8	3.1	0.9

[a] NR, not reported.
[b] EIA, enzyme immunoassay.

TABLE 4. Comparison of commercial assays for the detection of STEC[a]

Assay	STEC detection method	Analytical sensitivity		Clinical sensitivity (%)			Clinical specificity (%)		
		ST 1	ST 2	Stool	Broth	Colony[b]	Stool	Broth	Colony
Premier EHEC	Directly on stool; overnight incubation in MacConkey, GN, or EZColi broth; colonies	35 pg/ml	75 pg/ml	79	100		96	98	
ProSpeCT Shiga Toxin E. Coli (STEC)	Directly on stool; overnight incubation in MacConkey broth or TSB	52 pg/ml	126 pg/ml	87	99		98	99	
VTEC-Screen	Colonies	0.5 ng/ml	0.5 ng/ml			90			100

[a] Clinical sensitivity and clinical specificity values are in comparison with the cytotoxin assay. Abbreviations: GN, gram negative; ST 1 and ST 2, Shiga toxins 1 and 2, respectively; TSB, tryptic soy broth.
[b] Colony, overnight agar plate cultures.

screen for enteric pathogens. There is little justification for not employing the most sensitive method for detection of EHEC, at least in high-risk patients.

The *Manual of Commercial Methods in Clinical Microbiology* (11) lists four tests available for the detection of Shiga toxin in stool specimens. Since its publication, the RIM Immuno Shiga Toxin E. coli (STEC) Microplate Assay is no longer available from Remel, which now markets the ProSpecT Shiga Toxin E. coli (STEC) Microplate assay (Alexon-Trend, Ramsey, Minn.). There are no peer-reviewed publications evaluating this product; the manufacturer claims that it is similar to the Premier EHEC immunoassay (Meridian Bioscience, Inc., Cincinnati, Ohio). Both of these assays have been cleared by the FDA for use in the United States and can be performed with stool specimens directly or after overnight broth culture. The ProSpecT STEC assay employs polyclonal anti-Shiga toxin 1 and 2 antibodies to capture the toxin and horseradish peroxidase-labeled monoclonal mouse anti-Shiga toxin 1 and 2-labeled antibodies to detect the bound toxin. The Premier EHEC assay employs monoclonal anti-Shiga toxin 1 and 2 antibodies to capture the toxin and horseradish peroxidase-labeled polyclonal anti-Shiga toxin 1 and 2 to detect the bound toxin. A third assay, not submitted to the FDA, is the VTEC-Screen (Denka Seiken Co., Ltd., Tokyo, Japan). This reverse passive latex agglutination assay employs polymyxin B treatment of isolates to release verotoxins and kaolin to remove nonspecific reactions. The VTEC-Screen is performed on colony sweeps from overnight agar plate cultures and has a reported clinical sensitivity of 90% (2). The Premier EHEC assay can also be performed on colony sweeps. A comparison of these methods is presented in Table 4. The direct-specimen clinical sensitivities of the ProSpecT STEC assay and Premier EHEC are 87 and 79%, respectively. This is somewhat less than the overnight broth culture sensitivities of 99 and 100%, respectively, for the detection of STEC. In clinical studies, the sensitivity of the Premier EHEC has been reported to range from 91 to 100% (16, 18). When employing overnight broth culture to detect STEC, it is important to attempt to recover the isolate for serotyping and epidemiologic purposes. Performance of virulence factor-based tests, such as these, will allow us to document the true incidence of O157 and non-O157 STEC disease as well as the pathogenicity of non-O157 isolates.

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